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14. ABSTRACT

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Biological variation and diagnostic accuracy of dehydration assessment markers[1–4]

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ABSTRACT

Background: Well-recognized markers for static (one time) or dynamic (monitoring over time) dehydration assessment have not been rigorously tested for their usefulness in clinical, military, and sports medicine communities.

Objective: This study evaluated the components of biological variation and the accuracy of potential markers in plasma, urine, saliva, and body mass (B_m) for static and dynamic dehydration assessment.

Design: We studied 18 healthy volunteers (13 men and 5 women) while carefully controlling hydration and numerous preanalytic factors. Biological variation was determined over 3 consecutive days by using published methods. Atypical values based on statistical deviations from a homeostatic set point were examined. Measured deviations in body fluid were produced by using a separate, prospective dehydration experiment and evaluated by receiver operating characteristic (ROC) analysis to quantify diagnostic accuracy.

Results: All dehydration markers displayed substantial individuality and one-half of the dehydration markers displayed marked heterogeneity of intraindividual variation. Decision levels for all dehydration markers were within one SD of the ROC criterion values, and most levels were nearly identical to the prospective group means after volunteers were dehydrated by 1.8–7.0% of B_m. However, only plasma osmolality (P_{osm}) showed statistical promise for use in the static dehydration assessment. A diagnostic decision level of 301 ± 5 mmol/kg was proposed. Reference change values of 9 mmol/kg (P_{osm}), 0.010 [urine specific gravity (U_{sg})], and 2.5% change in B_m were also statistically valid for dynamic dehydration assessment at the 95% probability level.

Conclusions: P_{osm} is the only useful marker for static dehydration assessment. P_{osm}, U_{sg}, and B_m are valid markers in the setting of dynamic dehydration assessment. *Am J Clin Nutr* 2010;92:565–73.

INTRODUCTION

Healthy humans maintain homeostatic control of the balance of body fluids by physiologic and behavioral adaptations (1, 2). However, when fluids are limited, illness strikes, or there is exposure to extreme environments, the accumulation of fluid deficits can threaten homeostasis, health, and performance (3, 4). The efficacy of any assessment marker (5) for detecting disturbances in homeostasis depends critically on the nature of body fluid losses (6–8). Clinical dehydration is a state of hypertonic hypovolemia that occurs in response to a net loss of hypotonic body fluids (9–11). The rise in extracellular tonicity is a hallmark clinical feature of dehydration that provides diagnostic distinction from isotonic or hypotonic hypovolemia (6–8, 10). Dehydration predictably modulates renal function and urine composition in accordance with the deficit of body water (12). Thus, the fundamental principles of body fluid regulation provide the framework for using plasma osmolality (P_{osm}) (2, 10, 13, 14), urine osmolality (U_{osm}) (15–17), urine specific gravity (U_{sg}) (14, 15), urine color (U_{col}) (15), and, possibly, saliva osmolality (S_{osm}) (18, 19) as body fluid markers of dehydration. Similarly, because humans maintain a relatively stable total-body water pool, despite diverse factors that affect water requirements (eg, climate, activity, and dietary solute load) (2, 20), acute changes in body mass (B_m) may be used to accurately measure dehydration across both clinical and sports medicine disciplines (8, 21–25).

When the balance between fluid intake and output is carefully controlled experimentally, a consensus exists as to what constitutes euhydrated values for plasma and urine (4, 23) and surrogate B_m stability (23, 24). In contrast, it is well appreciated (1, 20) that modest perturbations in body fluid balance may be well tolerated and difficult to detect biochemically until some functional threshold ($\geq 2\%$ B_m) (4) is breached. For example, dehydration thresholds (in mmol/kg) of 586 (26), 716 (17), 830 (14), and 1052 (15) have all been proposed for U_{osm}. Because dehydration was experimentally assured across studies, strong population heterogeneity may be responsible for the apparent discordance of values. Indeed, there can be substantial heterogeneity in healthy individuals with respect to homeostatic control of the body fluid balance (1, 27), and the reference interval of typical population body fluid concentrations is wide (28). In accordance with a statistical distribution theory (29), the degree of overlap between typical and atypical values will determine the nosologic sensitivity of any dehydration assessment marker (29–32).

The degree to which any marker can correctly classify an individual as dehydrated can be described by its diagnostic quality or accuracy (33). Although good diagnostic accuracy is a prerequisite for any marker to have a practical value, it is inadequate alone for describing the potential usefulness or application of a test (33). Additional explanatory power can be gained with knowledge of the biological variability that underlies the population reference intervals. Very widely applied methods (29, 31) for isolating the analytic (CV_A), intraindividual (CV_I), and interindividual (CV_G) components of variation in body fluids can be used to understand the diagnostic usefulness (33) of a static value from one individual compared with a population-based reference interval (29, 31, 32). In addition, data on biological variation allow interpretation of the temporal nature of homeostatic control (34, 35) so that the importance of any dynamic change in serial measurements in an individual can be understood in longitudinal terms (29–31, 35). A knowledge of variation can also afford cautious assignment of probabilistic decision levels (29) and may even provide the necessary framework (29, 31) for determining the elusive gold standard marker for dehydration (5). Although simple reports of CV_I or CV_G can be found for P_{osm} (1), U_{osm} (17), U_{sg} (36), and B_m (24), few studies (37–40) have used the necessary methodology (29, 31) for extracting appropriate decision-making information. A study of the biological components of variation for multiple dehydration markers measured simultaneously from the same set of subjects is absent from the literature (41).

Components of biological variation (29, 31) for potential markers in plasma, urine, saliva, and B_m were evaluated to understand their usefulness in relation to static and dynamic dehydration assessments. The diagnostic accuracy (33) of dehydration markers was quantified separately by using prospective and comprehensive dehydration experiments. The latter experiment complemented the former experiment by providing actual physiologic measurements of dehydration against which the validity of probabilistic decision levels could be evaluated. A limited diagnostic characterization of dehydration markers is available, (26) and several unique markers have, to our knowledge, never been characterized (15, 18). This information holds important potential for reducing the effect and complications of dehydration across broad medical disciplines (4, 6–8, 10, 42, 43).

SUBJECTS AND METHODS

Subjects

Eighteen active soldier volunteers took part in this 2-wk study [13 men and 5 women; age (mean ± SD): 24 ± 4 y; B_m: 78.2 ± 12.1 kg; height: 172.2 ± 9.2 cm; body mass index (in kg/m^2): 26.4 ± 3.9]. All volunteers passed the Army Physical Fitness Test within the previous 6 mo and received a general medical clearance before participation: thus, all volunteers were considered physically fit and healthy. The use of alcohol, dietary supplements, and any medication other than an oral contraceptive was prohibited. No female volunteer was or became pregnant during the course of the study. Volunteers were provided informational briefings and gave voluntary, informed, written consent to participate. Investigators adhered to Army Regulation 70–25 and US Army Medical Research and Materiel Command

Regulation 70–25 on the use of volunteers in research. The US Army Research Institute of Environmental Medicine Human Use Review Committee approved this study.

Experimental design

Each volunteer completed 7 d of testing over 2 wk. The study was broken into 2 phases. In phase I, 3 consecutive days of testing were used to study biological variation. In phase II, 2 consecutive days of testing were completed twice separated by 1 wk.

Phase I

One day before the start of testing, an informational briefing was given to provide a detailed study schedule, to make anthropometric measurements, and to provide instructions regarding fluid intake. To achieve a euhydrated state before the first day of testing, volunteers were given 3.0 L fluid to consume over 24 h in addition to ad libitum beverage consumption and habitual occidental dietary practices. Volunteers were instructed to consume a premeasured 1.0 L water between waking and 1800 and another 2.0 L prepackaged sports drink for consumption between 1800 and 2200. It was also estimated that food intake would provide an additional 0.6 L fluid each day (2), which would bring daily fluid-intake totals to ≈3.6 L (2). Physical exercise was permitted but was restricted to a short list of allowable activities and work durations. No food or drink was permitted between 2200 and 0600 the next morning (8-h fast). All measurements began at precisely 0630 after a 30-min opportunity for personal hygiene. This procedure was repeated 3 times over as many consecutive days to establish the day-to-day biological variation of several markers while volunteers were euhydrated. In all, 54 measures of P_{osm}, U_{osm}, S_{osm}, U_{sg}, U_{col}, and B_m were planned in phase I.

Phase II

The daily procedure used in phase I was repeated again in phase II on day 1 and followed by a return to the laboratory in the early evening for dehydration and follow-up measurements on day 2. Dehydration was achieved by performing 3 to 5 h of work and rest cycles (50 min of work and 10 min of rest) that included treadmill (1.56 m/s; 4–7% grade) or cycle ergometer exercise (85–120 W) inside an environmental chamber set to 40°C and 20% relative humidity with a 1-m/s laminar wind flow. The purpose of the exercise-heat exposure was to increase body heat storage and induce sweating (hypotonic fluid loss) to achieve a state of hypertonic hypovolemia (9–11). The goal for dehydration spanned a functionally important range of 2.0–7.0% of B_m (2, 4, 20). After exiting the environmental chamber, volunteers were provided with a small, standardized meal (450 kcal; 57% carbohydrate, 30% fat, 13% protein, and 450 mg Na$^+$) and 0.2 L water or apple juice. No additional food or water was permitted, and volunteers were kept in supervised housing until morning. The phase II procedure was performed twice. After the measure of dehydration markers on the following morning, volunteers were rehydrated by oral and/or intravenous fluids to return B_m to within 1% of the euhydrated baseline. Measurements of P_{osm}, U_{osm}, S_{osm}, U_{sg}, U_{col}, and B_m were made on each day for a planned total of 36 euhydrated and dehydrated samples in phase II.

The sequence of events for all measurements remained consistent throughout the study. Upon reporting to (phases I and II of day 1) or awakening at the laboratory (phase II of day 2), volunteers voided a urine sample at 0630 and were weighed nude. Then, volunteers sat quietly for 30 min and remained seated while having their blood drawn, after which they provided a saliva sample. To examine the potential effect of simple oral artifacts (ie, food, drink, tobacco, and gum) on S_{osm} measures (23), a subset of 4 dehydrated volunteers provided an additional saliva sample ($n = 8$ samples) 1 min after a 10-s mouthrinse with 50 mL water.

Analytic measures

Plasma volume and body fluid osmometry

A 3-mL sample of venous blood was collected without stasis in lithium-heparin tubes (Sarstedt Inc, Newton, NC). Blood samples were centrifuged, and plasma was separated for analysis without delay (44). The first-void morning urine was collected in a sterile, inert polypropylene cup (Tyco Healthcare Group, Mansfield, MA), and a small aliquot was transferred into a 1.5-mL polypropylene cryule vial (Wheaton, Milville, NJ) and briefly vortexed before an analysis of osmolality. The remaining sample volume was analyzed for U_{sg} and U_{col}. A 2-mL sample of unstimulated whole saliva was collected into a single-use polypropylene Falcon tube (Voigt Global Distribution Inc, Lawrence, KS) and centrifuged before analysis. Plasma and saliva samples were centrifuged at $3500 \times g$ at $4°C$ for 10 min.

P_{osm}, U_{osm}, and S_{osm} were measured by freezing-point depression on 3 dedicated osmometers (Fiske Micro-Osmometer, model 210; Fiske, Norwood, MA) and always by the same 3 technicians assigned to the study. In addition to daily calibrations, each osmometer was calibrated in triplicate by using standards within the dedicated ranges for plasma (290 mmol/kg), saliva (50 mmol/kg), and urine (850 mmol/kg) before each new biological sample. The fluid samples were also run in triplicate, and the median value was taken as final. If any of the intrasample triplicate fluid measures differed by >1.0%, the median of 5 samples was used. This approach was recommended on the basis of the ordinate scale of the readings (44), and the desired imprecision was based on instrument resolution and the potential physiologic importance of small fluctuations ($\geq 1.0\%$) in P_{osm} to hormonal fluid regulation (12, 27). Plasma tonicity (effective osmolality) (13, 45) reflects the potential for osmotic water movement across cell membranes and should not include solutes that contribute to osmolality without affecting tonicity, such as plasma urea (13, 45). However, even after exclusion of major osmotically active moieties such as sodium, glucose, and urea, other ionic solutes may still account for deviations of ≥ 5–10 mmol/kg between measured osmolality and calculated (effective) tonicity (13, 45–47), regardless of what formula is chosen (45). This observation may explain, in part, why sodium alone is not an ideal dehydration assessment marker (2, 23). Therefore, the direct measure of P_{osm} was considered synonymous with total plasma tonicity in this study of healthy volunteers, despite the small plasma urea contribution to osmolality but not tonicity.

U_{sg} and U_{col}

After a small aliquot of urine was used for osmolality determinations, U_{sg} was measured in duplicate for each specimen with a refractometer (1110400A TS Meter; AO Reichert Scientific Instruments, Keene, NH) that was calibrated from 1.00 to 1.03 against liquid preparations of known relative density (mass/volume). U_{col} was determined in a naturally lit room against a standardized 8-point color chart produced by Armstrong et al (15). A single technician performed all U_{sg} and U_{col} analyses to avoid between-observer variability. A blinded, triplicate analysis of 3 specimens (9 observations) on a single day was used to test single-rater reliability for U_{col}.

B_m and percentage dehydration

In response to exercise-heat exposure, water (ie, sweat and urine) volume and B_m losses were considered equivalent (1 mL = 1 g) (22) after correction for carbon exchange and respiratory water loss, which was estimated at 2 g/min during heat exposure (21). The level of dehydration was calculated from the corrected change in nude B_m over 24 h (from 0630 on day 1 to 0630 on day 2) and expressed as a percentage of starting B_m. The starting 0630 nude B_m used in the denominator of the calculation was considered normal for an individual if it was within $\pm 1.0\%$ of his/her individual phase I mean (24), which was labeled as euhydrated on the basis of previous 24-h fluid consumption (2), and subsequently confirmed as euhydrated on the basis of meeting at least one additional plasma or urine concentration criterion (4, 23). The imprecision of the platform scale used (model WSI-600; Mettler Toledo, Toledo, Ohio) was checked daily at 25, 70, and 95 kg, and ± 50 g was considered an acceptable performance.

CV_A

The widely accepted methodology (29, 31) for generating and applying data on biological variation was used. In the case of assessing CV_A, the third approach described by Fraser and Harris (31) was used based on the instability of samples, freeze-thaw concerns, and the typical approach to osmolality assessment (44). Thus, the analytic imprecision (CV_A) reported for osmolality (P_{osm}, U_{osm}, and S_{osm}) represented the between-batch analytic imprecision by using osmolality control standards (average CV of 18 triplicate measures). The CV_A reported for all other markers was determined from the average CV of 3 duplicate (U_{sg} and B_m) or triplicate (U_{col}) calibration or specimen samples, respectively. All CVs were calculated as below:

$$(SD/mean) \times 100 \qquad (1)$$

Preanalytic variation (in vitro and in vivo) (29, 31) was minimized to the greatest extent possible. Therefore, it was assumed that biological variation around a homeostatic set point could be attributed to factors largely independent of preanalytic influences. The menstrual cycle phase could not be standardized and was accepted as one intrinsic source of biological variation.

Biological variation

One representative measurement, which was made daily for each volunteer, provided 54 potential P_{osm}, U_{osm}, S_{osm}, U_{sg}, U_{col}, and B_m values for analysis. No outliers were detected by using Reed's criterion with an exclusion ratio $\geq 1:3$. Gaussian data distributions were confirmed by the Shapiro-Wilk test; thus, all data are

expressed as means ± SDs. The total CV_I and CV_G were computed by analysis of variance according to published methods (31).

With the use of the 3 components of variation, the usefulness of a static measure against population-based reference intervals and the magnitude of dynamic changes required for statistical significance were determined. The ratio

$$(CV_A^2 + CV_I^2)^{1/2}/CV_G \qquad (2)$$

[ie, the index of individuality (II)] was calculated whereby a low value (<0.60: high individuality) indicated a low usefulness of population-based reference intervals, whereas a high value (>1.40: low individuality) indicated a high usefulness of population-based reference intervals (31, 32, 48, 50), particularly when an unusual result was repeated for verification (48). The magnitude of dynamic change required to make a difference statistically significant [ie, the reference change value (RCV)] was calculated as

$$2^{1/2} \times 1.65 \times (CV_A^2 + CV_I^2)^{1/2} \qquad (3)$$

for a one-sided test with a 95% probability level, which was consistent with a "very likely" clinical dehydration scale (29, 49). However, the RCV was not valid if the CV_I was heterogeneous in volunteers (31, 34). The index of heterogeneity (IH) was calculated as the CV of

$$(SD_A^2 + SD_I^2)^{1/2} \qquad (4)$$

divided by the theoretical CV if no heterogeneity existed:

$$[2/(n-1)]^{1/2} \qquad (5)$$

where n is the number of specimens collected from each volunteer (31, 34, 35). Because the number of replicates for each individual was precisely 3 in this study, the denominator was simplified to 1.0. Under the hypothesis of nonheterogeneity, the RCV was valid if

$$IH < \{1 + 2[1/(2n)^{1/2}]\} \qquad (6)$$

or 1.82 when n was set to 3 (31, 34, 35). We estimated that the potential for correlations between successive values to affect the RCV (29) was marginal because only RCV outcomes with a constant true variance (IH) were considered useful. A decision level for clinical judgments about dehydration was determined (29, 49, 50) by adding the RCV (units) to the euhydration grand mean. An SD for the decision level was calculated as below:

$$2^{1/2} \times SD_I \quad (50) \qquad (7)$$

Judgments concerning the usefulness of decision levels were based on the consideration of II. The validity of the RCV for dehydration markers was based on the consideration of IH. When valid, the RCV for each marker was expressed across 4 probability levels (80%, 90%, 95%, and 99%) to form a simple clinical likelihood scale (49) for dehydration monitoring.

Statistical analyses

The diagnostic accuracy of P_{osm}, U_{osm}, S_{osm}, U_{sg}, and U_{col} for detecting dehydration was determined by using receiver operating characteristic (ROC) analysis (GraphPad, 4.0; San Diego, CA). B_m was not included as a variable in the ROC analysis because the acute change in B_m itself serves to distinguish dehydration from euhydration (8, 22–25). The area under the ROC curve (AUC) represents the accuracy of the test for discriminating the presence of dehydration significantly better than chance (33). It also yields 2 valuable components of diagnostic accuracy, namely sensitivity and specificity or the fraction of true positive and true negative determinations, respectively. No precise tradeoff between sensitivity and specificity was assigned as long as both sensitivity and specificity were ≥80% (33), which represented odds of 4 to 1 in favor of a correct classification. The criterion value for optimal diagnostic performance was determined thereafter by the value with the highest combination of sensitivity and specificity.

When ≥2 ROC curves are generated by using the same set of subjects, a method analogous to the paired t test (51) can be used to compare performance diagnostics. Within each ROC analysis, the potential for repeated measures to reduce the SE and modestly inflate diagnostic accuracy was equitably ignored across all hydration measures (52). P_{osm} was used as the gold standard for comparison (2, 10, 13, 14). To control for α inflation of the 4 comparisons to P_{osm} (ie, U_{osm}, S_{osm}, U_{sg}, and U_{col}), Holm's method of sequential Bonferroni corrections for interdependent tests was used. S_{osm} rinse measures were analyzed by using repeated measures analysis of variance and Tukey's post hoc procedure. Significance was accepted at the 95% probability level. All data are presented as means ± SDs.

RESULTS

All 18 volunteers completed phases I and II of the study. All 54 possible samples were obtained on each measured variable in phase I with the exception of P_{osm}, for which there were 51 samples. In phase II, a complete set of 36 measurement pairs was obtained for ROC analysis on all measures except P_{osm} ($n = 33$) and S_{osm} ($n = 35$). Lost samples were due to phlebotomy problems with one volunteer, handling error, or an inability of one volunteer to produce enough saliva while dehydrated by ≈6.0% of B_m.

Phase I

A descriptive summary (mean ± SD) for the 3 d of euhydrated measurements in presented in **Table 1**. The group means were below (for U_{osm}, U_{sg}, U_{col}, and B_m) or slightly above (for P_{osm}) the euhydration cutoff values, but all of these variables were ≤1 SD of the cutoff, which did not suggest an overt body water deficit or dilution. The U_{osm}-to-P_{osm} ratio (2.1 ± 0.8) supported this conclusion as well (12, 16, 27). For variables where typical population intervals have been reported (ie, for P_{osm}, U_{osm}, U_{sg}) (28), results fell within these intervals.

TABLE 1
Plasma, urine, and saliva quantity estimates when euhydrated[1]

Quantity	Euhydration[2]	Measured ($n = 18$)	Range	Reference interval[3]
P_{osm} (mmol/kg)	≤ 290	292 ± 3[4]	284–298	275–295
S_{osm} (mmol/kg)	NA	71 ± 15	42–109	NA
U_{osm} (mmol/kg)	≤ 700	614 ± 205	205–1091	50–1200
U_{sg} (U)	≤ 1.020	$1.018 \pm 0\,0.006$	1.007–1.031	1.003–1.035
U_{col} (U)	≤ 4.0	3.8 ± 1.0	1.0–6.0	NA[5]
B_m (percentage change)	≤ 1.0	0.14 ± 0.48	-0.56–1.50	NA

[1] P_{osm}, plasma osmolality; S_{osm}, saliva osmolality; U_{osm}, urine osmolality; U_{sg}, urine specific gravity; U_{col}, urine color; B_m, body mass; NA, not applicable.
[2] Cheuvront and Sawka (23).
[3] Fischbach (28).
[4] Mean $\pm$ SD (all such values).
[5] Yellow, straw, and amber (no numerical anchor).

Euhydrated S_{osm} and U_{col} measures and typical percentage fluctuations in daily B_m were consistent with limited reports on a smaller scale (15, 18, 24).

The basic calculated components of variation (ie, CV_A, CV_I, CV_G) in this study are expressed as percentage CVs in **Table 2**. Rarely was a triplicate calibration CV for P_{osm}, S_{osm}, or U_{osm} >0.5%. Duplicate or triplicate measures for U_{sg}, B_m, and U_{col} were commonly identical. The CV_A for all measures was ultimately well below $0.5 \times CV_I$, as recommended (29, 31). The P_{osm} CV_A was only slightly smaller than previous reports (0.43–0.50) (37, 38) and a negligible (zero) value for B_m was also obtained by Widjaja et al (39). CV_I was smallest (0.4–1.3%) for P_{osm}, U_{sg}, and B_m and largest (9.5–30.9%) for S_{osm}, U_{osm}, and U_{col}. The CV_I and CV_G values for P_{osm} and B_m were also very comparable with Fraser et al (37) and Widjaja (39). The CV_I for U_{osm} was similar to Shephard et al (40), but the CV_G was higher.

CV_I was smaller than CV_G among all markers (Table 2), which was consistent with the general trend for most biological constituents of medical interest (29, 31, 32). As a result, the II was <1.4 for all markers and <0.60 for all but P_{osm} (0.90) and U_{col} (0.65). Only P_{osm} was considered to be of potential static dehydration assessment value (31, 32, 48, 50). For IH, only P_{osm}, U_{sg}, and B_m were considered to be of potential dynamic dehydration monitoring values (IH < 1.82) (31, 34, 35). All other variables displayed significant heterogeneity. The RCV, or magnitude of dynamic change in a serial result that would make a difference significant, is provided (**Table 3**) for 95% probability (dehydration very likely) as a percentage change in the

TABLE 2
Components of analytic variation (CV_A) and biological variation[1]

Quantity	CV_A	CV_I	CV_G
P_{osm} (mmol/kg)	0.4	1.3	1.5
S_{osm} (mmol/kg)	1.0	9.5	35.8
U_{osm} (mmol/kg)	0.7	28.3	57.9
U_{sg} (units)	Negligible	0.4	1.0
U_{col} (units)	Negligible	30.9	47.4
B_m (percentage change)	Negligible	1.1	26.6

[1] All values are percentages. CV_I, intraindividual variation; CV_G, interindividual variation; P_{osm}, plasma osmolality; S_{osm}, saliva osmolality; U_{osm}, urine osmolality; U_{sg}, urine specific gravity; U_{col}, urine color; B_m, body mass.

marker, as a unit change, and decision level. The unit RCVs at 4 probability levels for valid markers of dynamic dehydration monitoring only are presented in **Table 4**.

Phase II

Volunteers were dehydrated by $3.7 \pm 1.0\%$ of B_m (range: 1.8–7.0%). Individual and mean values for P_{osm}, U_{osm}, S_{osm}, U_{sg}, and U_{col} when subjects were euhydrated and dehydrated are depicted in **Figure 1** (A–E). Mean euhydrated values were nearly identical to measured values in Table 1 and again ≤ 1 SD of the suggested euhydration cutoff (23). The mean dehydration values were nearly identical to the 95% probabilistic decision levels (Table 3) with the exception of S_{osm}. The dotted lines in each panel represent the criterion values in **Table 5**, all of which were ≤ 1 SD of the probabilistic decision levels (Table 3). The U_{sg} criterion value was the same as that reported by Bartok et al (26), whereas P_{osm} and U_{osm} were substantially higher. Although S_{osm} was the only analyte that was statistically inferior to P_{osm}, the diagnostic accuracy of all analytes was significantly better than chance (Table 5), and the criterion value for each was balanced by a minimum 80% threshold for both sensitivity and specificity. In the S_{osm}-rinse study subgroup, euhydrated S_{osm} (88 ± 32 mmol/kg) increased significantly ($P < 0.05$) after dehydration (147 ± 88 mmol/kg) but dropped back to euhydrated values (81 ± 25 mmol/kg) 1-min after the mouthrinse even though volunteers remained dehydrated.

DISCUSSION

To our knowledge, this is the first study to carry out the determination and application of data on biological variation (29, 31) by using multiple dehydration assessment markers (23). Atypical values on the basis of statistical deviations from a homeostatic set point were examined alongside measured body fluid deficits induced by using a prospective dehydration study. We provide decision levels for multiple clinical markers of dehydration at the 95% probability level that can be used as a starting point to guide decision making for dehydration assessment across broad medical disciplines.

P_{osm} was the only body fluid marker studied that could potentially allow a correct diagnosis of dehydration from a static individual value framed against the typical, wide population-based reference interval. The II was high among all markers,

TABLE 3
Indexes derived from analytic and biological variation data[1]

Quantity	II	IH	RCV	RCV	Decision level
			%	U	
P_{osm} (mmol/kg)	0.90[2]	1.35[3]	3.1	9[4]	301 ± 5[5]
S_{osm} (mmol/kg)	0.27	9.53	22.2	16	86 ± 9
U_{osm} (mmol/kg)	0.49	28.32	65.9	404	1018 ± 245
U_{sg} (units)	0.44	0.43[3]	1.0	0.010[4]	1.028 ± 0.006
U_{col} (units)	0.65[2]	30.90	71.9	2.7	6.5 ± 1.7
B_m (percentage change)	0.04	1.08[3]	2.5	2.5[4,6]	2.5 ± 1.5[6]

[1] II, index of individuality; IH, index of heterogeneity; RCV, reference change value; P_{osm}, plasma osmolality; S_{osm}, saliva osmolality; U_{osm}, urine osmolality; U_{sg}, urine specific gravity; U_{col}, urine color; B_m, body mass.
[2] Between 0.6 and 1.4.
[3] Less than 1.82.
[4] Useful for dynamic dehydration assessment.
[5] Potentially useful for static dehydration assessment.
[6] 1.96 kg expressed as the percentage of the 78.2-kg group mean B_m.

indicated by a small ratio as can be seen in equation (2) and consistent with the general trend for most biological moieties of medical interest (29, 31, 32). Volunteers with a small II (<0.60) can experience a change in any dehydration assessment marker that is very atypical for them, but their values would still fall within the typical population-based reference interval (31, 32, 48, 50). When II is >1.40, atypical individual values will be seen with greater frequency. However, the probability of identifying an atypical individual result within a population-based reference interval increases rapidly as II exceeds 0.60 and approaches 1.00 (32), particularly if a confirmatory measurement is made (48). The II for P_{osm} (0.90) was very near the values of 0.93 and 1.40 reported by Fraser et al (37) and Jahan et al (38), respectively.

Although there is disagreement on how to interpret a P_{osm} between 295 and 300 mmol/kg (42), a P_{osm} decision level of 301 ± 5 mmol/kg offers a 95% probability of dehydration and was close to the group mean for dehydration achieved prospectively (304 ± 6 mmol/kg) (Figure 1A). This P_{osm} decision level also exceeds the upper 295 mmol/kg population-based reference limit (10, 28) and encompasses the sometimes proposed overt dehydration value of 300 mmol/kg (8, 42). The ROC criterion value of 297 mmol/kg (Figure 1A), which displayed high sensitivity and specificity for dehydration (Table 5), was ≤1 SD of the decision level and identical when an 80% probability level was applied to the RCV calculation (Table 4). When decision levels were calculated for S_{osm}, U_{osm}, U_{sg}, U_{col}, and B_m (Table 3), they were also very near the group means for

TABLE 4
Reference change value (RCV) and dehydration likelihood scale[1]

Probability	Unidirectional z score	Dehydration likelihood	RCV units		
			P_{osm}	U_{sg}	B_m
			mmol/kg	units	%
80%	0.84	Likely	5	0.005	1.3
90%	1.28	More likely	7	0.008	2.0
95%	1.65	Very likely	9	0.010	2.5
99%	2.33	Virtually certain	13	0.014	3.5

[1] Concept adapted from Fraser et al (49). P_{osm}, plasma osmolality; U_{sg}, urine specific gravity; B_m, body mass.

experimental dehydration and ≤1 SD of the ROC criterion values in Table 5. Although this seems to support a narrow range of values as good general starting points, II data negate their relevance for static dehydration assessment (31, 32, 48, 50) in high-risk treatment situations. However, a practical use in circumstances of lower-risk assessment may still be warranted (4, 5, 23).

Dynamic dehydration assessment was best accomplished by using P_{osm}, U_{sg}, and B_m (Table 3) on the basis of the prerequisite homogeneity of CV_I (31, 34, 35). Because of the study design and the number of specimens collected for each volunteer, the validity of the RCV was only accepted when IH was <1.82 (Table 3). Whether serial changes were calculated by using the 95% probability or some other level of confidence (Table 4), the corroborating data suggested that serial changes would be detectable whether they occurred over hours or days, and possibly even over several months (30). On the other hand, the objective assessment that CV_I was not constant for S_{osm}, U_{osm}, and U_{col} (Table 3) casts doubts on their use in this manner. The fact that U_{sg} was useful for serial measures may seem counterintuitive given that renal concentrating mechanisms produce strong relations among U_{sg}, U_{osm}, and U_{col} (15). However, strong relations contain substantial scatter, and U_{osm} may vary by as little as 81 (U_{sg}: 1.012) or as much as 533 mmol/kg (U_{sg}: 1.021) at any given U_{sg} measured across a broad range (1.005–1.028) (53). The categorical nature of the U_{col} chart led to measurement imprecision on the scale required for clinical usefulness, although it may still be of practical use for low-risk population assessment (15, 23). Importantly, any urine concentration measure must be interpreted carefully because disease states, time of day of urine collection (first morning void preferred), and drinking behaviors can all confound urine interpretation for hydration (4, 14, 15, 17, 23, 28, 53). S_{osm} results were similar to U_{osm} and U_{col} results when II and IH were concerned, and S_{osm} also performed to the minimum ROC standard (Table 5; Figure 1B). The potential effect of simple oral artifacts (ie, food, drink, tobacco, and gum) to mar the diagnostic usefulness of S_{osm} (18, 19) is also worrisome considering the subgroup S_{osm}-rinse outcomes.

The results of this study in young, healthy volunteers should have relevant application to military (43) and sports medicine (4) populations. Fraser et al (37) clearly showed that RCVs generated from young subjects can be applied to healthy older subjects (> 70 y) because biological variation does not change with age if health is maintained. Indeed, studies of biological variation require observations from a healthy population (29, 31). Thus, the RCVs of 9 mmol/kg (P_{osm}), 0.010 (U_{sg}), and 2.5% (B_m) could be used broadly for dynamic dehydration assessment as long as baseline values are within the typical population-based reference intervals. It is also possible that a P_{osm} of 301 ± 5 mmol/kg could be used as a decision level for static dehydration assessment within both a healthy and stable, but diseased, clinical population (29, 31). Because clinical assessment of an individual with an unknown health status involves the calculation of an osmolal gap (measured – calculated) (13), a P_{osm} of 301 ± 5 mmol/kg in the presence of a gap <10 mmol /kg would also indicate dehydration because the pathologic glucose or urea values (3 times upper reference limit) required to raise a P_{osm} above the decision level would be simultaneously ruled in or out by their direct measurement (13, 45). Because
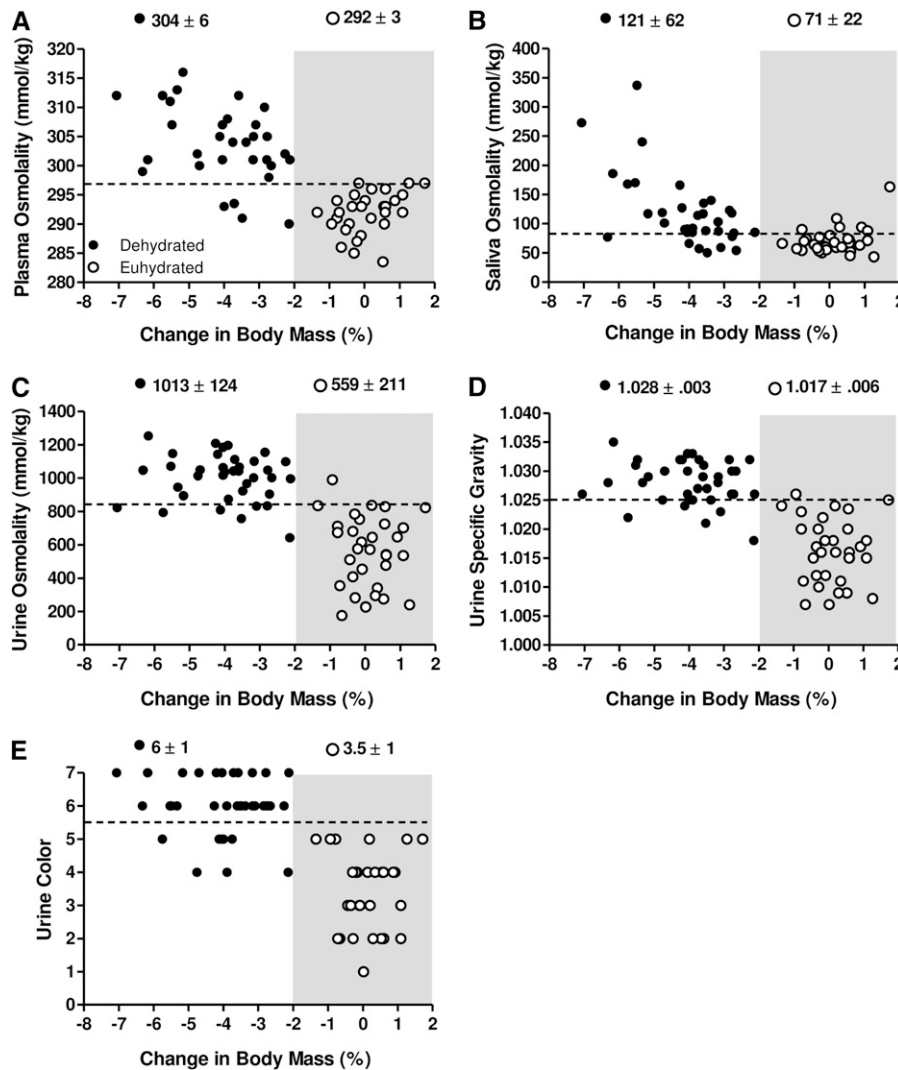
FIGURE 1. Individual and mean ± SD values of plasma osmolality (A), saliva osmolality (B), urine osmolality (C), urine specific gravity (D), and urine color (E) plotted as a function of change in percentage body mass when euhydrated (open circles) and dehydrated (closed circles). Data represent 33 (A), 35 (B), or 36 (C–E) pairs of observations. The dashed lines represent the criterion value determined by receiver operating characteristic analysis (Table 5). Areas beyond the gray zone represent hydration changes (dehydration) of increasing functional importance (2, 4, 20).

any unusual P_{osm} result is likely to be repeated, II >1.0 should decrease the number of false-positive confirmatory measures (48). A P_{osm} of 301 ± 5 mmol/kg would be valid only for diagnosing hypertonic hypovolemia (6, 7, 10, 13). A gold-standard method for diagnosing hypovolemia of isotonic or hypotonic origins remains elusive (7).

All biological variation and ROC analysis data were predicated on the fact that volunteers were euhydrated daily (phase I) and before dehydration was imposed (phase II). Euhydration can be supported on the basis of 24-h fluid intakes ≥3.0 L (2) and on the grounds that all volunteers were below euhydration cutoff values for ≥2 hydration measures each day (4, 23). The mean values for all volunteers were also never >1 SD below the cutoff, and the U_{osm}-to-P_{osm} ratio was not close to 1.0 (dilution) (12, 16, 27) or >3.0 (concentrated) as observed after a 14-h fast (53). However, the group mean for P_{osm} was slightly higher (292 ± 3 mmol/kg) (Table 1; Figure 1A) than the corresponding 290 mmol/kg euhydration cutoff. Many of the studies used to support the <290 mmol/kg cutoff used a testing methodology

whereby fluid was consumed by subjects in the morning before blood sampling. The grand mean P_{osm} for these studies was 284 mmol/kg (2). A P_{osm} in this range or lower would not alter the usefulness of the RCV, but it would reduce the sensitivity of the 301 ± 5-mmol/kg decision level. However, fewer than 2% of free-living people have a P_{osm} <285 mmol/kg, and most free-living people have a P_{osm} between 285 and 295 mmol/kg when they consume >3.0 L fluid each day (42). A P_{osm} <285 mmol/kg is also commonly associated with a U_{osm} <400 mmol/kg (27) and, therefore, results in a U_{osm}-to-P_{osm} ratio consistent with mild dilution. Indeed, fluid volumes vary (when reported), but data from Robertson et al (12) support a 1 mmol/kg drop in P_{osm} for each 175 mL water consumed after an overnight fast. There is substantial individual variation in the P_{osm} set point brought about by an even larger variation in the thirst threshold and arginine vasopressin sensitivity (27). Taken together, a P_{osm} of 292 ± 3 mmol/kg is a defensibly euhydrated value that reflects an overnight fluid-restriction methodology coupled with ordinary biological variation.

TABLE 5
Receiver operating characteristic curve analysis of a prospective dehydration experiment[1]

Quantity	Criterion value	AUC	Sensitivity	Specificity
			%	%
P_{osm} (mmol/kg)	297	0.95[2]	90	100
S_{osm} (mmol/kg)	83	0.83[2,3]	80	83
U_{osm} (mmol/kg)	831	0.98[2]	91	91
U_{sg} (units)	1.025	0.96[2]	89	91
U_{col} (units)	5.5	0.96[2]	81	97

[1] AUC, area under the curve; P_{osm}, plasma osmolality; S_{osm}, saliva osmolality; U_{osm}, urine osmolality; U_{sg}, urine specific gravity; U_{col}, urine color.

[2] Indicates AUC discriminates significantly better than chance.

[3] Indicates a significantly smaller AUC compared with the AUC for P_{osm}.

In conclusion, the sports medicine literature provides a consensus about what threshold values constitute euhydration by using a variety of dehydration assessment markers (4, 23). We add to this literature by providing decision levels for multiple body fluids that can be used as starting points for the diagnosis and treatment of dehydration. Values that occur between euhydration and dehydration represent the typical human variation in homeostatic set points because of biology (1, 20) as well as social (ie, diet) and environmental (ie, exercise and climate) influences (2). Currently, the P_{osm} provides the best potential measure for static dehydration assessment, whereas dynamic dehydration assessment is best accomplished by using P_{osm}, U_{sg}, and B_m. The use of ≥ 2 markers should provide added diagnostic confidence when serial measures are made (4, 23). These findings should be useful for clinical, military, and sports medicine communities.

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